MUTATIONS OF BACTERIAL VIRUSES AFFECTING THEIR HOST RANGE[1]

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Received July 8, 1944

INTRODUCTION

WHEN susceptible bacteria are spread on a solid culture medium with a large amount of a bacterial virus, complete lysis occurs after incubation, except for the appearance in some cases of colonies consisting of virus-resistant cells. These are the descendants of bacteria that had undergone a mutation from virus-sensitivity to virus-resistance prior to the action of the virus (LURIA and DELBRÜCK 1943). The virus-resistant bacteria do not adsorb the virus. Their resistance is generally specific, not extending to unrelated viruses. Conversely, when a virus is plated with a suspension of bacteria resistant to its action, it generally does not affect the bacteria; a uniform layer of bacterial growth results. We observed, however, that plating very large amounts of a virus with a resistant bacterial mutant strain occasionally results in the formation of a few clear "plaques"—that is, of a few virus colonies. From these plaques a new virus strain may be isolated that is active on the bacterial mutant resistant to the normal virus. A study of the origin of the new virus proved that it arises by mutation from the normal virus. A mutation of the virus can thus compensate for a mutation of the bacterial host. The present paper is concerned with the study of these virus mutations and of their relation to bacterial mutations.

Mutations affecting characters of bacterial viruses have been described before (GRATIA 1936a; BURNET and LUSH 1936). In 1929, SERTIC clearly recognized the occurrence of true breeding variants of bacterial viruses capable of attacking bacterial strains resistant to the original virus. He appears to have considered such variants as the result of an adaptation of the virus when in the presence of resistant bacteria.

EXPERIMENTAL

Material and basic findings

The material for the experiments described in this paper consisted originally of a strain of *Escherichia coli* **B** and of two viruses, α and γ, active on strain **B** (DELBRÜCK and LURIA 1942). Virus α gives large plaques, virus γ small plaques when plated with **B** on solid media. From strain **B**, a series of mutant strains can be isolated, some sensitive to virus α and resistant to virus γ, others sensitive to virus γ and resistant to virus α. These strains are obtained as secondary growths after lysis of **B** by virus γ or virus α. They are easily purified by repeated streak platings, and their resistance to one of the two viruses is

[1] Aided by a grant from the DAZIAN FOUNDATION FOR MEDICAL RESEARCH.

generally found to be complete: the virus is not adsorbed by the bacterial cells and does not grow in their presence.

We have adopted in this paper the convention of naming the mutant bacterial strains by the Roman letter corresponding to the original strain, followed by the Greek letter corresponding to the virus in the presence of which they have developed as secondary growth after lysis. For example, strain **B**α indicates a mutant from **B** isolated as secondary growth after **B** was lysed by virus α. This mutant will generally be resistant to virus α (unless otherwise specified). Strains isolated in the presence of the same virus, but different in some properties (colonial morphology, range of sensitivity, etc.), are distinguished by sub-indexes: **B**α_1, **B**α_2, · · · Mutants obtained from other mutants are named by adding the Greek letter corresponding to the virus in the presence of which the new mutant has been isolated: **B**$\alpha\gamma$, for example, will be a mutant from **B**α isolated after lysis of **B**α by virus γ, and will generally be resistant to both viruses α and γ. This notation offers the advantage that the name of each mutant strain mirrors its previous history, indicating the strain of origin and the virus, or viruses, whose selective action has brought about its isolation.

From strain **B**, one can easily isolate two mutants, **B**α_1 and **B**α_2, differing in some growth characteristics but both resistant to virus α and sensitive to virus γ. Very exceptionally, one obtains from **B** a mutant **B**γ, resistant to virus γ and sensitive to virus α.[2]

Plating very large amounts of virus γ with a suspension of cells **B**γ often produces some small, clear plaques, similar to the regular γ-plaques produced on **B**. The ratio of the number of plaques thus produced to the number of plaques produced by the same virus suspension when plated with strain **B** is generally very small (of the order of 10^{-7}, occasionally up to 10^{-5}). For a given suspension of virus, this ratio remains constant, at least for several months. The plaques produced on **B**γ being clear, they cannot be attributed to non-homogeneity of the bacterial suspension, with presence of some γ-sensitive cells: the action of a virus on a bacterial mixture partially sensitive results in the formation of plaques of turbid appearance, due to the growth of the resistant cells.

If some of the contents of one of the few plaques produced by a suspension of virus γ on **B**γ are picked up and immediately plated again with a suspension of **B**γ, a large number of plaques are obtained. This confirms the expectation that the plaque contained a large number of virus particles attacking **B**γ. By means of repeated one-plaque isolations and platings with **B**γ, a pure virus strain is obtained which can be grown in liquid cultures of **B**γ. This pure virus strain we called γ'; its properties will be described in the following section.[3]

It may be stated here that the presence of virus γ' has been found in concen-

[2] The strains **B**γ and **B**α_1 have been called A and C in previous papers (DELBRÜCK and LURIA 1942), in which the interest was focused on their use as indicator strains for plating mixed virus suspensions rather than on their mutational origin.

[3] Several of the experiments with virus γ' were done using a strain **B**$\gamma\alpha_2$ instead of strain **B**γ, because our stock of strain **B**γ (obtained from strain **B** in 1941) had recently, in the course of subculturing, become partially sensitive to virus γ. Strain **B**$\gamma\alpha_2$, derived from **B**γ before this occurred, behaved toward viruses γ and γ' exactly like the original strain **B**γ.

trated suspensions of virus γ prepared directly from one γ-particle (one γ-plaque transferred to a liquid culture of **B**, incubated until lysis, lysate filtered). This proves that virus γ' does not represent an initial non-homogeneity of the stock of virus γ, but arises from normal particles of virus γ.

We have said that at least two different mutants, $B\alpha_1$ and $B\alpha_2$, can be isolated after lysis of B by virus α. Plating any amount of virus α with bacteria $B\alpha_1$ does not result in any effect on the bacterial growth. We found, however, that plating very concentrated suspensions of virus α with cells of strain $B\alpha_2$, characterized by slow and limited growth on nutrient agar, often results in the formation of a few clear plaques. These plaques contain a true breeding virus α', active on strains B and $B\alpha_2$, but not on $B\alpha_1$. The properties of this virus α' will be discussed in a later section.

The culture media used in this study were: nutrient broth +0.5 per cent NaCl for liquid cultures; the same plus 1.1 per cent powdered agar for platings. All experiments were performed in water-baths or incubators at 37°C. The technique for growth curves of bacteria, growth experiments of the viruses, and experiments on interference between viruses have been described in detail in a previous paper (DELBRÜCK and LURIA 1942). The meaning of certain terms used hereafter, however, may be recalled: *constant period* = minimum time between infection of a bacterium by virus and its lysis with virus liberation; *burst size* = average yield of virus particles per lysed bacterium; *infective center* = anything that produces one plaque when plated with sensitive bacteria. An infective center, therefore, can be either a free particle of virus or a bacterium infected by the virus; both of them will give just one plaque when plated with sensitive bacteria; *efficiency of plating* = the ratio between the number of plaques produced by a virus suspension in a given plating and the maximum number of plaques which that suspension can give when plated under optimum conditions. The latter has been shown to correspond very closely to the actual number of active virus particles present (DELBRÜCK and LURIA 1942).

Properties of virus γ'

Each one of the plaques obtained by plating a suspension of virus γ with $B\gamma$ is capable of yielding a strain of virus γ', which can be purified by repeated platings with $B\gamma$ and one-plaque isolations, since normal virus γ does not grow on bacteria $B\gamma$. After isolation and purification, virus γ' can be grown in liquid cultures of either bacteria B or $B\gamma$; filtrates of such cultures yield stable stocks. Whether grown on strain B or on strain $B\gamma$, the particles of virus γ' show exactly the same properties, as hereafter described.

The plaques produced by virus γ' on B are small, sharp edged, and indistinguishable from those produced by virus γ. Those produced on $B\gamma$ can be distinguished with some experience mainly because of their slightly larger size.

An interesting feature of virus γ' is that of giving a smaller number of plaques when plated with strain $B\gamma$ than with B. The efficiency of plating on $B\gamma$ varies from 0.2 to 0.6 of that on B. It seemed important to investigate whether or not this difference in the efficiency of plating was due to non-

homogeneity of the particles of virus γ'. It could be imagined that the γ'-particles in reproducing gave origin to a certain proportion of particles of type γ, and therefore active on **B** only. This was excluded by experiments of the following type: Virus γ' was plated with cells **B** in amounts that gave a few hundred plaques. After incubation, the contents of each of 20 plaques were picked up with a needle, inoculated into separate samples of a suspension of cells **B**γ, and immediately plated. All the plates showed numerous plaques, proving that all the plaques produced by the suspension of virus γ' on **B** actually contained virus γ'.

Another conceivable possibility was that a certain proportion of the particles in a suspension of virus γ' produced virus γ' when growing on **B**, but were not themselves capable of attacking cells of type **B**γ. This possibility was excluded by experiments of the following kind, directed to prove that all particles in a suspension of virus γ' can attack **B**γ in liquid media: A definite amount of virus γ', after titration with both bacteria **B** and **B**γ, was added to an excess of growing cells **B**γ. After allowing a few minutes for adsorption (10 minutes, that is, much less than the constant period; see below), the mixture was divided into two portions. One portion was centrifuged for titration of the free virus in the supernatant, the other portion was tested for the number of infective centers, by plating separately with **B** and with **B**γ. Since no liberation of new virus had yet taken place, each infective center represented either a free virus particle or an infected bacterium. If those particles of virus γ' that give plaques on **B** and not on **B**γ are actually unable to attack **B**γ, they will remain free and not appear as infective centers on **B**γ. If, on the contrary, they can be adsorbed by **B**γ, they will account for a part of the infected bacteria. As such, they are likely to appear as infective centers on **B**γ, because each infected bacterium later liberates a large number of virus particles, thereby increasing the chances of plaque formation. This will result in an increase of the number of infective centers relative to the original input of virus as measured by plating with **B**γ. Table 1 shows the results of three such experiments.

It is seen that the number of infective centers on **B**γ after adsorption by **B**γ

TABLE 1

The number of infective centers of virus γ' after adsorption by cells **B**γ.

	EXPERIMENT NO. 13		EXPERIMENT NO. 14		EXPERIMENT NO. 16	
	PLATING WITH **B**	PLATING WITH **B**γ	PLATING WITH **B**	PLATING WITH **B**γ	PLATING WITH **B**	PLATING WITH **B**γ
Virus input per cc	27×10^6	7×10^6	35×10^6	7×10^6	11×10^6	3×10^6
Free virus per cc	8×10^6	2.7×10^6	8×10^6	2.5×10^6	2.7×10^6	1.1×10^6
Infective centers after adsorption per cc	28×10^6	27×10^6	35×10^6	28×10^6	11.5×10^6	10.5×10^6

TABLE 2

Growth of virus γ′ on bacterial strains **B** *and* **Bγ** *in broth at 37°C.*

	STRAIN **B**	STRAIN **Bγ**
Generation time of the bacteria	19 minutes	24 minutes
Virus adsorbed in 5 minutes (7×10^7 bacteria/cc)	80%	less than 10%
Virus adsorbed in 5 minutes (2×10^8 bacteria/cc)	over 90%	40%
Constant period	21 minutes	21 minutes
Burst size	95–130	40–60

is almost as high as the number of infective centers on **B**. The differences are partly accountable for by the free virus, partly by sampling errors. These experiments prove that all particles in a suspension of virus γ′ are capable of attacking cells **Bγ** —that is, they are all γ′-particles.

The low efficiency of plating of virus γ′ with **Bγ** must, therefore, be due to some reason other than those considered above. The most likely explanation is that it is a result of the very low adsorption rate of the virus by these cells, as shown below. Many particles of virus may either remain unadsorbed on the agar or be adsorbed by bacteria too late to produce a fully developed, visible plaque. Any such explanation is bound to be tentative, in view of our incomplete knowledge of the process of plaque formation.

The interaction of virus γ′ with bacteria **B** and **Bγ** was studied by means of adsorption experiments and "one-step growth" experiments (DELBRÜCK 1942). The results are given in table 2. The interaction of virus γ′ with bacteria of strain **B** is in every respect similar to that of virus γ (DELBRÜCK and LURIA 1942). Strain **Bγ** adsorbs virus γ′ much more slowly than strain **B**. In order to obtain measurable adsorption, the experiments with **Bγ** had to be done with older cultures, containing more cells than those used for **B**. The cells in such cultures are likely to be of smaller size, and this makes the values for the adsorption rates and also for the burst size not strictly comparable. An experiment with cells **B** grown to reach the same concentration as used for **Bγ**, however, showed a burst size of 95—that is, not so low as for **Bγ**.

It is interesting to notice that, whereas the bacterial strain **Bγ** has a longer generation time than **B**, virus γ′ grows on **Bγ** with the same constant period as on **B**. The burst size, however, is smaller. The mutation **B**→**Bγ** involves, besides the change in virus sensitivity, changes in the rate of bacterial division and in the yield of virus γ′ per cell.

Interference of virus γ with the growth of virus γ′

Interference between different bacterial viruses growing on the same host has been described and found to conform to the general rule that one cell liberates only virus of one type (DELBRÜCK and LURIA 1942; DELBRÜCK 1944). It was concluded previously that interference also occurs between particles of the same virus strain: when a cell is attacked by several particles of the same strain

(multiple infection), the result is the same as with single infection, as if only one particle could grow in a cell. Direct proof of this "self-interference" was difficult to obtain, since the offspring of a virus particle is indistinguishable from that of another particle of the same virus. Interference with the growth of virus γ by an excess of ultraviolet inactivated virus γ (LURIA and DELBRÜCK 1942) was an indirect confirmation of the occurrence of self-interference.

The availability of virus γ', identical with virus γ in its behavior toward cells of strain **B**, but traceable by its activity on strain **B**γ, offered an opportunity for further study of self-interference. The experiments were done by adding both viruses γ and γ', the former in excess, to cells **B**, then using **B**γ as a plating indicator for virus γ'. If virus γ interferes with the growth of virus γ', a bacterium infected first with virus γ, then with virus γ', will not liberate any virus γ' and will not give a plaque when plated with **B**γ. A loss of infective centers will result.

We give here the data from such an experiment, in which the results were made more clear by the use of anti-virus serum to eliminate the free virus. DELBRÜCK (1944) has found that anti-virus serum inactivates free virus but generally does not affect the growth of virus particles already adsorbed by bacteria.

A culture of **B** was divided into two portions. At time zero one portion received an excess of virus γ, and one minute later a smaller amount of virus γ'. The other portion received only virus γ'. After five minutes, the cultures were diluted into tubes containing a dilution of anti-γ serum sufficient to reduce the amount of free virus (both γ and γ') to about 1 per cent in three minutes (see a later section). At the eighth minute the mixtures were highly diluted into broth to stop the action of serum, and the infective centers of virus γ' were measured by plating with **B**γ at 12 and 17 minutes—that is, before liberation of new virus had taken place. Other platings were done to determine the amounts of free viruses. The results may be summarized as follows:

	I portion	II portion
Bacteria/cc	8×10^7	8×10^7
Absorbed virus γ/cc	80×10^7	——
Adsorbed virus γ'/cc	2×10^7	2×10^7
Infective centers γ'/cc	0.2×10^7	2×10^7

It is seen that in this experiment about 90 per cent of the bacteria infected with both viruses γ and γ' failed to liberate any virus γ'. Other experiments, in which anti-virus serum was not used, gave results of the same type, although the presence of free virus γ' made the loss of infective centers less conspicuous. If we are justified in considering virus γ' as identical to virus γ in its action on strain **B**, we can view these experiments as a direct proof of the occurrence of interference between similar virus particles adsorbed by the same cell.

Properties of virus α'

Virus α', as isolated from the few plaques obtained by plating virus α with **B**α_2, is indistinguishable from virus α in its activity on bacteria of strain **B**. The

plaques are identical, and so is the growth in liquid medium: constant period 13 minutes, burst size 110–130 in broth at 37°C. Plated with bacteria of strain $B\alpha_2$, a suspension of virus α' gives a smaller number of plaques than with B. The efficiency of plating is 0.25–0.7. The plaques are of smaller and more variable size. In liquid medium, bacteria $B\alpha_2$ adsorb virus α' extremely slowly, so that reliable adsorption measurements are difficult to obtain. The constant period for growth in broth at 37°C is 13 minutes, similar to the growth on B. The burst size is difficult to determine with any degree of accuracy, because the presence of large amounts of unadsorbed virus disturbs the calculation of the number of infected bacteria. From an experiment in which at least a great part of the free virus was eliminated by means of anti-serum, we obtained for the burst size a minimum value of 55.

There are some indications that strains of virus α' independently isolated may differ from one another. The size of the plaques produced on the same bacterial strain is sometimes different. This point has not yet been further investigated.

The mutational origin of viruses α' and γ'

The presence of virus α' in suspensions of virus α and that of virus γ' in suspensions of virus γ are demonstrated by plating a large amount of the suspensions with bacteria resistant to the normal virus. We observe only the end result—that is, the appearance of a few plaques containing a new type of virus. Several alternative modes of origin of the new virus are *a priori* conceivable. Hypothesis 1: There is a small finite probability that, when plated with *resistant* bacteria, a normal virus particle succeeds in attacking one of the resistant cells; when this happens, then the particle will give rise to a virus strain capable of attacking the bacteria resistant to the original virus. Hypothesis 2: There is a small finite probability that a normal virus particle in a culture of virus growing on *sensitive* bacteria mutates, becoming hereditarily capable of attacking the resistant bacteria. Hypothesis 3: There are in a sensitive bacterial culture some exceptional, abnormal bacteria which, when infected by a normal virus particle, produce virus of the new type instead of the normal type.

As far as hypotheses 1 and 2 are concerned, the situation is similar to that encountered in the study of virus-resistant bacteria from virus-sensitive bacterial strains, and analogous considerations apply (LURIA and DELBRÜCK 1943).

According to hypothesis 1, on the one hand, the number of virus particles that succeed in giving plaques on the resistant bacteria should be proportional to the number of virus particles tested. This will be true whether these particles come from the same virus culture or from different cultures, since the particles that produce plaques on the resistant bacteria are normal particles at the time of plating, and the probability of producing a plaque is assumed to be uniform for all particles. If we test a large number of samples each containing the same amount of normal virus, the numbers of plaques produced on the resistant bacteria should show only the fluctuations due to the sampling er-

ror. These numbers should therefore show a Poisson distribution (variance = mean).

According to hypothesis 2, on the other hand, the new type of virus particles stem from mutations occurring during the growth of normal virus on sensitive bacteria, prior to the test. If a mutation occurs before the growth of the virus is completed, the mutant particle will multiply on the normal bacteria and give rise to a clone of mutant particles. The earlier a mutation occurs, the larger the clone will be. If we test a large number of samples, each containing the same amount of normal virus, the result will be different, depending on whether the samples come from the same virus culture or from different virus cultures. If we test different samples of the same culture, we shall again find a Poisson distribution of the number of plaques produced on resistant bacteria. If, however, we test a series of similar virus cultures, all started with a few sensitive bacteria and a few normal virus particles and all containing the same final amount of virus, the numbers of plaques produced will show a distribution with a variance much higher than the mean, because of the presence of clones of mutant particles.

The situation in the case of bacterial viruses is more complicated than in the case of bacteria. The virus particle multiplies by infecting a sensitive bacterium, which, after a latent period, liberates a hundred or more new particles. The mechanism of multiplication of the virus inside the bacterial cell is not known. If the new type of virus arises by mutation during the growth of the normal virus, the distribution of the number of mutant particles will depend on the modalities of the growth of the virus inside the cell.

As far as hypothesis 3 is concerned, if the new type of virus is produced by some abnormal cells, these may be expected to liberate a full burst of particles of the new type. Therefore, we should find that in each virus culture the particles of the new type occur in clones averaging the burst size.

The three hypotheses thus lead to different predictions regarding the distribution of the numbers of plaques produced on resistant bacteria by a series of similar cultures of virus. Accordingly, experiments were undertaken to determine this distribution. In these experiments, a small number of bacteria of strain **B** (about 10^8/cc) were added to a broth suspension of either virus α or virus γ containing 10^3–10^4 particles/cc. The mixture was immediately divided into portions of 0.2 or 0.5 cc and these were incubated at 37°C. Upon incubation, the cultures of virus γ always remained clear (complete lysis, due to the rarity of the mutation $B \rightarrow B\gamma$). Cultures of virus α generally also gave complete lysis; only occasionally a few cultures prepared under such conditions showed secondary growth of resistant bacteria. This is explained by the fact that generally in such cultures complete lysis takes place before the bacteria reach a titer high enough to render the occurrence of mutations $B \rightarrow B\alpha$ likely. If exceptionally a mutation $B \rightarrow B\alpha_1$ occurs, the bacteria $B\alpha_1$, resistant to both viruses α and α', grow to saturation. If a mutation $B \rightarrow B\alpha_2$ occurs, the mutant cells grow to saturation if no α'-particle is present. If α'-particles are present, they are adsorbed by the cells $B\alpha_2$ when the concentration of the latter is high enough, and the result is a culture containing a large amount of virus α', com-

TABLE 3

Distribution of the numbers of plaques produced on $B\alpha_2$ by a series of similar cultures of virus α.

EXPERIMENT NO.	112	113	114	115a	115b
Number of cultures tested	20	34	87	11	40
Volume of each culture, cc	.2	.5	.5	.5	.5
Virus α per culture	1.2×10^8	7×10^8	8.9×10^8	9×10^8	7.8×10^8
NUMBER OF PLAQUES PRODUCED ON $B\alpha_2$	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES
0	16	9	3	2	6
1	1	5	2	2	1
2	3	6	1	2	2
3	0	2	2	1	4
4	0	1	2	0	1
5	0	1	4	0	1
6	0	1	3	0	1
7	0	0	2	0	2
8	0	1	1	0	1
9	0	1	2	0	0
10	0	0	1	0	0
11–20	0	3	11	1	9
21–50	0	1	14	2	10
51–100	0	1	13	1	1
101–1000	0	1	21	0	1
over 1000	0	1	5	0	0
Average per culture	.35	45	125	13.7	15.6
Variance	.57	1200	45,000	570	380

parable to its titer of virus α. In our experiments all these possibilities were actually realized: we found several cultures with growth of $B\alpha_1$, one culture with growth of $B\alpha_2$, and one culture completely lysed but containing such a large amount of virus α' as to suggest that virus α' had multiplied on a secondary growth of $B\alpha_2$.

After incubation, the completely lysed cultures were tested for the amount of virus α (or virus γ) and for the number of plaques produced on α-resistant (or γ-resistant) bacteria. For the latter purpose, the whole contents of each culture were plated according to the technique devised by GRATIA (1936b) and by HERSHEY et al. (1943). The results are given in tables 3 and 4. The amounts of virus α (or virus γ) represent averages; the individual counts of normal viruses in different cultures within each experiment never vary more than can be accounted for by the sampling errors. In considering the counts from the platings with the resistant bacteria, we must remember that for both virus α' and virus γ' the efficiency of plating is low and slightly variable from one experiment to another. The plaque counts should be too low by a factor corresponding to the efficiency of plating. It is worth recalling that this factor, if constant within one experiment, should not affect the type of distribution *if*

TABLE 4

Distribution of the numbers of plaques produced on B_γ *by a series of similar cultures of virus* γ.

EXPERIMENT NO.	27	28a	28b	28c	28d	29
Number of cultures tested	20	9	9	9	9	40
Volume of each culture, cc	.2	.2	.2	.2	.2	.2
Virus γ per culture	20×10^8	16×10^8	15×10^8	22×10^7	6×10^7	15×10^7
NUMBER OF PLAQUES PRODUCED ON B_γ	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES	NUMBER OF CULTURES
0	1	2	3	6	8	25
1	0	0	0	1	0	8
2	0	2	4	2	0	2
3	1	1	1	0	0	3
4	2	2	0	0	0	2
5	1	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	2	0	0	0	0	0
9	1	0	0	0	0	0
10	0	0	0	0	0	0
11–20	4	2	0	0	0	0
21–50	3	0	0	0	0	0
51–100	2	0	0	0	1	0
101–1000	2	0	1	0	0	0
over 1000	1	0	0	0	0	0
Average per culture	190	4.8	55	.55	8	.72
Variance	215,000	24	27,000	.82	540	1.4

this is a Poisson one. The experimental distribution will be derived from the theoretical one by multiplying the mean by the efficiency of plating and will still be a Poisson distribution.

It is immediately seen in tables 3 and 4 that the fluctuations of the numbers of plaques produced on the resistant bacteria are much higher than could be accounted for by the sampling errors. The variance is generally much higher than the mean, in accord with the expectation from the hypothesis that the new virus arises by mutation from the normal virus.

Another result evident in tables 3 and 4 is the presence of a large proportion of low values, well below the value of the burst size. This suggests that a bacterium may liberate a mixture of normal and mutant viruses and seems to exclude the possibility that the new virus be produced in full bursts by some abnormal bacteria, according to hypothesis 3. The new virus particles arise by mutation in the course of the multiplication of normal virus inside the cell.

It would now be desirable to compare the experimental distribution of the number of mutant virus particles with the distributions to be expected accord-

ing to various conceivable mechanisms of multiplication of the virus inside the bacterial cell, thus proving or disproving the correctness of each of these mechanisms. This comparison, however, is hampered by the low efficiency of plating of both the mutant viruses α' and γ'. Some of the theoretical distributions are not completely known, and the effect on them of the low efficiency of plating cannot be predicted. This is true, for instance, of the distribution encountered in the study of bacterial mutations (LURIA and DELBRÜCK 1943), which would apply with some modifications to our case if the virus multiplied in the cell as bacteria multiply in a culture. Besides, the efficiency of plating may not be exactly constant even within each experiment, and its variations may affect any distribution. Further discussion of this point is postponed until experimental data obtained under conditions in which the efficiency of plating is equal to one will be available. Experiments in this direction are now in progress.

Similar considerations make it pointless to attempt figuring values for the rates of mutation from the data in tables 3 and 4. Obviously, the correct mode of calculating mutation rates depends on the unknown mechanism of virus multiplication.

Antigenic relation of normal and mutant viruses

Bacterial viruses injected into the animal body stimulate the production of antibodies, of which the best known property is their specific virus-neutralizing power. Antisera against viruses α, γ, and γ' were prepared by repeated injections into white rabbits, until the homologous titer had reached a satisfactory level. Cross-inactivation tests were then performed. It was found that, within the experimental precision, a serum neutralized virus α and virus α', or virus γ and virus γ', with the same titer and at the same rate. As an example, we give the values of the fractional rate of neutralization (HERSHEY et al. 1943) obtained by testing the same dilutions of various sera on normal and mutant viruses:

Serum anti-γ : $k_\gamma = 1.3$; $k_{\gamma'} = 1.0$.
Serum anti-γ': $k_\gamma = 0.8$; $k_{\gamma'} = 0.8$ (approximate values).
Serum anti-α : $k_\alpha = 0.3$; $k_{\alpha'} = 0.35$.

No cross-inactivation was found between viruses α and α' on the one hand and viruses γ and γ' on the other hand. For virus γ', it was found that both its activities on B and on Bγ were neutralized at the same rate.

These results clearly show that, whatever structural differences exist between a virus and a mutant derived from it, they are not revealed by serological tests, at least of this rather simple nature.

The virus mutations in relation to the bacterial mutations

Arising by mutation from viruses α and γ, viruses α' and γ' acquire the ability to attack bacterial strains that by mutation have lost their sensitivity to the original virus. The virus mutations compensate for the bacterial mutations. The mutation $\gamma \rightarrow \gamma'$ is complementary to the mutation B$\rightarrow$Bγ; the mutation $\alpha \rightarrow \alpha'$ is complementary to the mutation B$\rightarrow$Bα_1. That the compensation

is not complete is shown by the fact that the mutant viruses are adsorbed by the mutant bacteria more slowly than by the normal bacteria. One may say that the affinity of the mutant viruses for the mutant bacterial strains is poorer than for strain B.

Strain Bγ is sensitive to virus α and to virus γ'. Does the mutation $\gamma \rightarrow \gamma'$ involve the acquisition by the virus of the same structural configuration that enables virus α to attack strain Bγ? If so, mutants from strain Bγ which are resistant to virus α should also be resistant to virus γ'. It was easy to isolate from Bγ several α-resistant mutants, called B$\gamma\alpha$; all of them proved perfectly sensitive to virus γ' (fig. 1). Sensitivity to viruses α and γ' cannot, therefore, be due to the same structural configuration.

It was more difficult to isolate bacterial mutants stably resistant to virus γ'. Eventually, we obtained from strain Bα_1 a mutant strain B$\alpha_1\gamma'$ that proved resistant to virus γ and to virus γ', and, surprisingly enough, sensitive to virus α. The sensitivity of strain B$\alpha_1\gamma'$ to virus α was unexpected, since this strain was a mutant from strain Bα_1, which was completely resistant to virus α. The mutation B$\alpha_1 \rightarrow$B$\alpha_1\gamma'$, therefore, involved loss of sensitivity to viruses γ and γ', but gain of sensitivity to virus α.

It may be added that from strain B$\alpha_1\gamma'$ a mutant B$\alpha_1\gamma'\alpha$ can easily be obtained, resistant to all three viruses α, γ, and γ' (fig. 1).

Let us now consider the situation concerning virus α'. The mutation $\alpha \rightarrow \alpha'$ involves gain of ability to attack the α-resistant mutant Bα_2. This ability is not due to the same configuration which enables virus γ to attack Bα_2, as proved by the fact that virus α' also attacks a mutant strain B$\gamma\alpha_2$, resistant to virus γ. Differences in the configurations of virus α' and virus γ' are brought out by their relations to the mutants B$\gamma\alpha_1$ and B$\alpha_1\gamma'$ (fig. 1). The remarkable point here is that the mutation $\alpha \rightarrow \alpha'$ compensates for only certain mutations of α-sensitive bacteria—namely, B$\rightarrow$Bα_2 and B$\gamma\rightarrow$B$\gamma\alpha_2$, but not for the mutations B$\rightarrow$Bα_1 or B$\gamma\rightarrow$B$\gamma\alpha_1$. It is worth mentioning that both strains Bα_2 and B$\gamma\alpha_2$ belong to the group of "small colony" mutants that grow poorly and slowly on nutrient agar, whereas Bα_1 and B$\gamma\alpha_1$ are normal colony formers. This is an example of a modification of the cultural, physiological properties of the bacterial cells correlated with the mutational change to virus-resistance.

The results summarized in figure 1 indicate that various bacterial mutations leading to resistance to unrelated viruses are generally independent of each other. For instance, the same two mutations (sub-1 and sub-2) leading to resistance to virus α (with or without resistance to virus α' and change in cultural characters) can occur either in strain B or in strain Bγ, in spite of the fact that the latter has already undergone a mutation toward resistance to virus γ.

Figure 1 also shows the occurrence of α'-resistant strains, all of which are also resistant to virus α.

The results of this section show that some, if not all, mutational changes in virus sensitivity by our bacterial strains can be compensated for by complementary mutations of the viruses. It is possible that in cases of bacterial mutations for which complementary virus mutations have not been found (for ex-

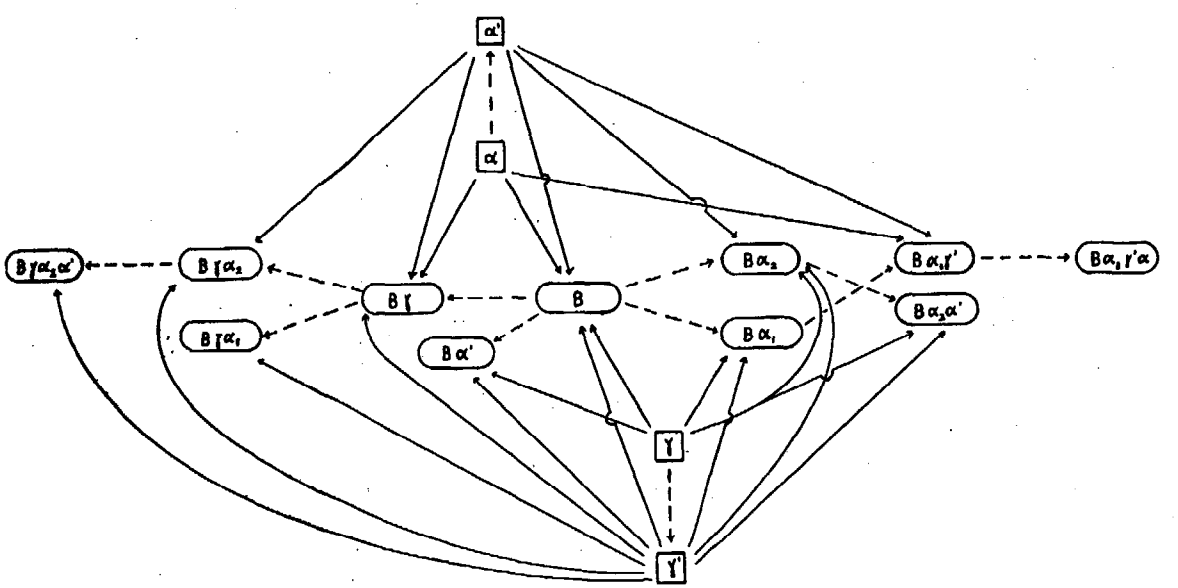

FIG. 1.—The sensitivity relations between mutant bacterial strains and virus mutants. Broken arrows indicate mutations. Solid arrows indicate activity of a virus on a bacterial strain.

ample, B→Bα_1), the virus mutations occur with a frequency too low to make them detectable in virus suspensions of the usual titers.

It is clear from the results obtained with viruses α and α' that loss of sensitivity to a given virus can be brought about, in the same bacterial strain, by different mutations, leading to differences in sensitivity to another virus closely related to the first.

DISCUSSION

The results described above demonstrate that, whereas bacteria can be altered by mutation in their susceptibility to bacterial viruses, the latter can in turn acquire by mutation the ability to attack new bacterial strains. The reverse change, by which a virus particle would lose by mutation the capacity of attacking a certain bacterium, might conceivably occur, but would be difficult to demonstrate, except in cases where it occurred very frequently.

The changes in virus properties are here called "mutations" because of their apparently spontaneous and random occurrence, of their transmission to the offspring, and of their stability. The same may be said of the bacterial mutations affecting virus-sensitivity. In making any analogy with the process of gene mutation in plants and animals, we should not forget the lack of any direct evidence of the presence, in bacteria or viruses, of "genes" in the sense of discrete material units, whose existence in higher organisms is proved by linkage studies.

As for the structural changes involved in the virus mutations, we have seen that the serological tests failed to reveal any difference between original and mutant strains. For the time being, our only basis for attempting to understand the structural changes involved in the mutations is the infectivity of the viruses for different bacterial strains.

The virus-host interaction involves as first step a process of specific adsorption. The specificity of the adsorption of a virus by a bacterium is generally conceived as due to the presence of "receptors" for the virus on the bacterial surface (see BURNET 1930). Adsorption is conditioned by complementarity of the surface structures of the receptor and of the virus, which enables them to fit together. Since the mutant bacteria resistant to a virus do not adsorb that virus, we may assume that the bacterial mutation causes a change in the receptors. It has long been known (BURNET 1930) that changes in virus-sensitivity of bacterial strains are often accompanied by changes in the antigenic make-up of their surface, and some of the antigens have been supposed to be responsible for virus adsorption.

The fact that a change in the surface structure of a bacterium can be compensated for by an independent change in the virus particle suggests that the changes involved are relatively small, possibly limited to simple stereochemical rearrangements, suppressing or restoring the complementarity of the surface structures. The fact that normal and mutant viruses are serologically indistinguishable speaks in favor of this conception. It is interesting to recall that STANLEY (1943) and his collaborators found that strains of tobacco mosaic virus, supposed to be closely related to each other, showed serological relation-

ship, common structural pattern in X-ray diffraction studies, and similarity of gross chemical composition, but showed differences in finer chemical composition.

The low rate of adsorption of the mutant bacterial viruses by their new hosts may be attributed to a less satisfactory fitting of the surface structures of virus and bacterium, lowering their affinity.

A possible alternative explanation must be mentioned—namely, that the acquired ability of a mutant virus to be adsorbed by a new host may be due to a change in only one out of a number of surface structures of the virus particle. This would explain the smaller adsorption rate of the mutant viruses by the mutant bacteria, while the rate of adsorption by the normal bacteria remains the same as for normal viruses.

The evolutionary implications that the results discussed above present for the system bacterium-virus are of some interest. Because of the lytic activity of most bacterial viruses, a bacterial strain is doomed to destruction once it has come in contact with a virus active upon it. The only chance of survival of the bacterial strain is the occurrence of mutations to virus-resistance. As we have seen, however, this occurrence does not necessarily protect the strain, because its mutation to virus-resistance may be compensated for by an independent complementary mutation of the virus. In certain cases (SERTIC 1929) parasitism may be maintained by two parallel series of complementary mutations in the host and the parasite.

Mutations of bacterial viruses enlarging their host range need not always be limited to activity upon closely related bacterial strains, but may conceivably render a virus active on strains belonging to different species. It is interesting, from the standpoint of bacterial taxonomy, that while bacterial viruses may be active on species belonging to different genera, chiefly within the family Enterobacteriaceae, no virus has ever been found to be active on members of different families.

SUMMARY

From two bacterial viruses, α and γ, two new viruses α' and γ' were isolated, differing from α and γ by their ability to attack bacterial strains which by mutation had become resistant to virus α or to virus γ.

An analysis of the distribution of the particles of a new virus in a series of similar cultures of normal virus proved that the new virus arises by mutation from the particles of normal virus in the course of their growth on sensitive bacteria.

Each mutant is indistinguishable from its parent virus in serological properties and in its activity on the common bacterial host. The latter property was utilized to study the interference between similar virus particles. The results confirmed the conclusion that only one of the infecting particles succeeds in growing in each bacterial cell.

The mutant viruses are poorly adsorbed by their new hosts. Their growth on these was investigated.

The sensitivity of a series of bacterial mutants to viruses α, α', γ, and γ' was studied. It was found that resistance to virus α may be brought about in the same bacterial strain by different mutations. These lead to differences in sensitivity to the related virus α' and in other physiological properties.

Bacterial mutations leading to resistance to a mutant virus lead also to resistance to the parent virus.

Bacterial mutations leading to resistance to unrelated viruses generally prove independent; the same mutation can occur in strains with a different history of previous mutations. One exception to the independence of various mutations was found. A bacterial strain resistant to viruses α and α' reverted to sensitivity as a consequence of a mutation to resistance to viruses γ and γ'.

ACKNOWLEDGMENTS

Some of the experiments reported in this paper were done in the summer of 1944 in the Department of Biology, VANDERBILT UNIVERSITY, Nashville, Tennessee. The author expresses his thanks to VANDERBILT UNIVERSITY for the hospitality and for a grant-in-aid, and to DR. M. DELBRÜCK for many helpful discussions.

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